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1/77

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Request for grant of a patent

The Patent Office

28 MAY 1999

Cardiff Road
Newport
Gwent NP9 1RH

1.	Your reference	CAH /4906
2.	Patent application number *(The Patent Office will fill in this part)*	**9912639.3**
3.	Full name, address and postcode of the or of each applicant *(underline all surnames)*	Britannia Pharmaceuticals Limited 41-51 Brighton Road Redhill Surrey RH1 6YS
	Patents ADP number *(if you know it)*	
	If the applicant is a corporate body, give the country/state of its incorporation	UK
4.	Title of the invention	"Improvements in and relating to treatment of respiratory conditions"
5.	Name of your agent *(if you have one)* "Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*	Abel & Imray 20 Red Lion Street London WC1R 4PQ
	Patents ADP number *(if you know it)*	174001

6.	If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number	Country	Priority application number *(if you know it)*	Date of filing *(day/month/year)*

7.	If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application	Number of earlier application	Date of filing *(day/month/year)*

8.	Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:* *a) any applicant named in part 3 is not an inventor, or* *b) there is an inventor who is not named as an applicant, or* *c) any named applicant is a corporate body.*	Yes

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Continuation sheets of this form	
Description	21
Claim(s)	6
Abstract	1
Drawing(s)	1 + 1

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Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	

11. I/We request the grant of a patent on the basis of this application.

Signature	Date
Abel & Imray	28th May 1999

12. Name and daytime telephone number of person to contact in the United Kingdom — Ceris Humphreys — 0171 242 9984

Improvements in and relating to treatment of respiratory conditions

This invention relates to medicaments for use in the treatment of asthma and to delivery devices including the medicaments.

It has been estimated that asthma affects between 4 and 10 percent of the population, causing distress and alarm to both sufferers and bystanders. Asthma attacks appear to be precipitated in many cases by a number of factors such as exercise or pollutants in the inspired air. Other agents such as pollen and airborne particles may predispose an asthma sufferer to an attack by sensitising the airways. This has led to the belief that effective treatment should include administration of drugs which reduce the sensitivity of asthma sufferers to allergens or which neutralise the allergic reaction.

The lungs and airways of non-asthmatics may contain a natural protective barrier which prevents pollutants and other potential irritants from reaching receptors which would otherwise produce an acute attack. Studies have suggested that it is possible to simulate in the lungs of asthma sufferers the situation in normal lungs by causing surface-active phospholipids (SAPL) to bind to the tissue surface of the lungs, thereby reducing the number of receptors exposed to noxious stimuli and reducing the broncho-constrictor reflex.

SAPLs are used clinically for the treatment of respiratory distress syndrome (RDS) in neonates. In this role, it has been assumed that the SAPL functions by reducing the high surface tension forces at the air-water interface within the alveoli, thereby reducing the pressure needed to expand the lungs, see Bangham et al., Colloids &

Surfaces, 10 (1984), 337 to 341. Thus, commercially available formulations of SAPL have been designed to spread rapidly over an air-aqueous interface, thereby reducing what is otherwise a very high surface tension of water.

Limited clinical studies have been carried out to determine the effect of commercial SAPLs marketed for treatment of RDS in neonates on asthmatic subjects, - see Kurashima *et al* Jap. J. Allergol 1991; 40, 160. This paper reported some amelioration of bronchoconstriction in asthmatic adults using an SAPL obtained by extraction from bovine lungs. In another study on children, also using an SAPL obtained from bovine lungs, no significant changes in lung function or histamine response were found, - see Oetomo et al - American Journal of Respiratory and Critical Care Medicine 153; 1996, page 1148.

EP 0 528 034A describes the use of pulmonary surface active material as an ingredient of an antiasthmatic, which is in the form of a liquid or suspension for injection or spraying into the patient's air way.

The present invention provides a delivery device for administering to a patient by inhalation a medicament for the prevention or treatment of asthma, the delivery device containing a medicament comprising a surface active phospholipid composition which is in finely divided powder form and comprises a first component which is a phospholipid capable of binding to lung tissue and a second component which is a phospholipid capable of enhancing the spreading of said first component, the delivery device being arranged for delivery of at least one individual inhalable dose, the or each individual dose comprising said first and second components in a combined amount of 10mg.

The invention also provides a delivery device for administering to a patient by inhalation a medicament for

the prevention or treatment of asthma, the delivery device containing a medicament, the medicament being in finely divided powder form and comprising a first component consisting of one or more phosphatidyl cholines and a second component consisting of one or more compounds selected from the group consisting of phosphatidyl glycerols, phosphatidyl ethanolamines, phosphatidyl serines, phosphatidyl inositols and chlorestyl palmitate, the delivery device being arranged for delivery of at least one individual inhalable dose, the or each individual dose comprising said first phospholipid component and said second component in a combined amount of at least 10mg.

It is believed that the combinations of compounds administrable in finely divided powder form by the device of the invention have two important effects:-

First, the medicament has surfactant properties, which enable it to spread rapidly over the surfaces of the lungs and air passages. It is an important feature of the present invention that the medicament is in the form of a powder, that is, it is in solid form. The "dry" surfactant has a high surface activity. It is believed that, on contact of the first component with the mucous within the lungs, the presence of the second component results in a lowering of the melting point of the first component, promoting rapid spreading of the first component over the liquid-air interface as a thin film at body temperature. For example, the normal melting temperature of dipalmitoyl phosphatidyl choline, which is a preferred first component, is about 40°C, that is, above the normal body temperature. When used in combination with a suitable second component, such as a phosphatidyl glycerol, however, the melting point of the dipalmitoyl phosphatidyl choline can in effect be reduced to below the normal body temperature.

Second, once the medicament is *in situ* over the surfaces of the lungs and air passages, a component of the composition is thought to migrate across the mucous layer enabling a thin hydrophobic lining or coating to be adsorbed onto the tissue surface. Thus, over and above the surface tension reducing properties mentioned above, the medicament of the invention is believed to provide a protective effect by virtue of the adsorbed layer. In binding to the epithelium, the phospholipid may mask the irritant receptors which elicit the bronchorestrictor reflex, that is, which cause narrowing of the bronchi.

As indicated above the delivery device is arranged for dispensing individual inhalable doses of medicament each individual dose comprising at least 10mg of said first and second components. Whereas phospholipids have been disclosed previously as adjuvants in certain forms of delivery device, the amounts of phospholipid administered in a dose by those previously disclosed devices have been much smaller than those required according to the present invention. In fact, it is preferred in accordance with the present invention for each individual dose to comprise at least 25mg, and more especially at least 40mg of said first and second components. The first and second components are substantially non-toxic, and the upper limit of the dosage may therefore in general be selected having regard to convenience taking into account matters such as, for example, the comfort of the patient and/or design parameters of the device. In general, however, the device will be such that it can deliver doses of up to 1000mg, advantageously up to 500mg, preferably up to 200mg, and especially up to 100mg.

The medicament used in the device of the invention is in finely divided solid form. "Finely divided" as used

herein means that the material has a particle size distribution which is such that at least a major proportion by weight of the particles are small enough to enter into a patient's airways and, preferably, deep into the lungs when inhaled. In practice, the first and second components preferably each have a particle size distribution which is such that not less than 90%, by weight, of the particles of those components in combination, and more preferably of each of the first and second components, have a particle size of not greater than 10μm, and especially of not greater than 5μm. Advantageously, the median particle size of the combined first and second components, and more preferably of each of the first and second components is not more than 10μm, and preferably not more than 5μm. The median particle size may be less that 3μm, for example, about 1.2μm. It may be desirable in some circumstances for the particles to have a median particle size of at least 0.5μm. The size of the particles may be calculated by laser diffraction, or by any other method by which the aerodynamic diameter of particles can be determined. "Median particle size" as used herein means mass median aerodynamic diameter ("MMAD"). The MMAD may be determined using any suitable method, for example, using a Multi-Stage Liquid Impinger in accordance with the method described in European Pharmacopoeia (supplement 1999) 2.9.18 (Aerodynamic assessment of fine particles). Alternatively, the size distribution of the particles may be characterised by their volume mean diameter (VMD). Advantageously, the VMD is not more than 10μm, for example not more than 5μm, and preferably less than 3μm. Finely divided dry powders of this kind (which may be described as fumed powders) can be adsorbed onto the surfaces of lung tissue and are believed, in use, to become bound to the epithelium.

A finely divided solid mixture of said first and second components may be obtained by size reduction of larger particles by any suitable size reduction method preferably before mixing. Preferably, the first component of the medicament comprises one or more compounds selected from the group consisting of diacyl phosphatidyl cholines. Examples of suitable diacyl phosphatidyl cholines (DAPCs), are dioleyl phosphatidyl choline (DOPC); distearyl phosphatidyl choline (DSPC) and dipalmitoyl phosphatidyl choline (DPPC). Each of those compounds appears to be capable of forming a thin film or coating on surfaces of the lungs. Most preferably, the first component is DPPC.

The second component may comprise one or more compounds selected from the group consisting of phosphatidyl glycerols (PG); phosphatidyl ethanolamines (PE); phosphatidyl serines (PS); phosphatidyl inositols (PI) and chlorestyl palmitate (CP).

Phosphatidyl glycerol (PG) is believed to be capable of binding to lung tissue and possibly enhancing the binding of the first component and is, therefore, a preferred second component. PG is also a preferred second component because of its ability to form with the first component a very finely-divided, dry powder dispersion in air.

The medicament advantageously comprises a diacyl phosphatidyl choline and a phosphatidyl glycerol. The phosphatidyl glycerol is advantageously a diacyl phosphatidyl glycerol. The acyl groups of the phosphatidyl glycerol, which may be the same or different, are advantageously each fatty acid acyl groups which may have from 14 to 22 carbon atoms. In practice, the phosphatidyl glycerol component may be a mixture of phosphatidyl glycerols containing different acyl groups. The phosphatidyl glycerol is expediently obtained by synthesis

from purified lecithin, and the composition of the acyl substituents is then dependent on the source of the lecithin used as the raw material. It is preferred for at least a proportion of the fatty acid acyl groups of the phosphatidyl glycerol to be unsaturated fatty acid residues, for example, mono- or di- unsaturated C18 or C20 fatty acid residues. Preferred acyl substituents in the phosphatidyl glycerol component are palmitoleoyl, oleoyl, linoleoyl, linolenoyl and arachidonoyl. The medicament preferably comprises dipalmitoyl phosphatidyl choline and phosphatidyl glycerol, with the phosphatidyl moiety of the phosphatidyl glycerol advantageously being obtainable from the phosphatidyl moiety of egg lecithin.

The first and second components may be present in a weight ratio of from 1:9 to 9:1. Advantageously, the proportion by weight of the first component exceeds that of the second component. Preferably, said first component and said second component are present in a weight ratio of from 6:4 to 8:2. At a weight ratio of about 7:3, the mixture spreads rapidly at a temperature of 35°C or above. DPPC can be prepared synthetically by acylation of glycerylphosphorylcholine using the method of Baer & Bachrea - Can. J. Of Biochem. Physiol 1959, 37, page 953 and is available commercially from Sigma (London) Ltd. The PG may be prepared from egg phosphatidylcholine by the methods of Comfurions *et al*, Biochem. Biophys Acta 1977, 488, pages 36 to 42; and Dawson, Biochem J. 1967, 102, pages 205 to 210. When co-precipitated with DPPC from a common solvent such as chloroform, PG forms with DPPC a fine powder which spreads rapidly over the surfaces of the airways and lungs. The most preferred composition of the invention contains DPPC and a phosphatidyl glycerol derived from egg phosphatidyl choline and having a mixture of C16, C18 (saturated and

unsaturated) and C20 (unsaturated) acyl groups. One form of that composition is obtainable from Britannia Pharmaceuticals Ltd., 41-51 Brighton Road, Redhill, Surrey, under the trade mark "ALEC". For use in the device of the present invention, however, it is preferred for the particle size of the mixture to be less than that of "ALEC" in the form in which it is currently obtainable commercially. To obtain a mixture in which the particle size is suitable for use in the device of the invention, the phospholipid components may be dissolved in a suitable solvent, for example ethanol, the solution filtered and vacuum-dried, and the solid product size-reduced to obtain particles of the desired size. During size-reduction, care should be taken to protect the mixture from moisture, oxygen, direct heat, electrostatic charge and microbial contamination.

Advantageously the medicament further comprises one or more respiratory drugs including but not limited to drugs selected from the group consisting of $ß_2$-agonists, steroids, cromones and leukotriene receptor antagonists. The medicament may include one or more said respiratory drugs in an amount of up to 10 parts, especially up to one part by weight per hundred parts by weight phospholipid. It will be appreciated that the respiratory drug or drugs should be present in such an amount that each dose delivered by the device contains an effective amount of the drug or drugs.

The medicament may comprise a $ß_2$-agonist which may be terbutaline, a salt of terbutaline, for example terbutaline sulphate, or a combination thereof or may be salbutamol, a salt of salbutamol or a combination thereof. Salbutamol and its salts are widely used in the treatment of respiratory disease. The active particles may be particles of salbutamol sulphate. Long-acting $ß_2$ adrenoceptor agonists

may be present, for example, formoterol, salmeterol, and salts thereof.

The medicament may comprise an antimuscarinic drug, for example ipatropium bromide.

The medicament may comprise a steroid, which may be, for example, beclomethasone dipropionate, budesonide, triamcinolone acetonide or may be fluticasone. The medicament may comprise other prophylactic drugs, including cromones, for example, sodium cromoglycate or nedocromil. The medicament may include a leukotriene receptor antagonist.

The medicaments of the invention have the further advantage that the first and second components may be of synthetic origin. It has been found undesirable to expose asthmatic patients to proteins of animal origin, because such proteins can have a sensitising effect on such patients, and thus the use of synthetic material has considerable advantages over the use of surfactants of animal origin that may contain animal protein.

Because it is desirable in the present invention to achieve a relatively long term adsorption of the medicament on the lung surface, it is highly desirable that the medicament (or any active components) should not break down in the environment of the lungs. One of the factors which will reduce the life of a lining or coating will be the presence of enzymes, such as phospholipase A, capable of digesting DPPC and/or PG. Such enzymes only attack the laevorotatory (L) form, which constitutes the naturally occurring form. Therefore, the medicament should preferably contain the dextrorotatory (D) form or at least comprise a racemic mixture, which is obtained by synthetic routes. Suitable dispersion devices may employ a propellant such as a halocarbon to form the gas stream and may include a

tapered discharge nozzle baffle or a venturi to accelerate particles through a discharge nozzle, and to remove oversized particles. Suitable halocarbons include hydrofluorocarbons, hydrofluorochlorocarbons and fluorochlorocarbons having a low boiling point, such as those marketed under the trade mark "Freon". The medicament may be packaged with a propellant in a pressurised aerosol container within the inhaler. Other inhalers have an impeller which mixes the powder into an air stream and delivers the powder-laden air into the patient's airways - see, e.g. US 5,577,497.

A preferred method and apparatus for administering the medicament involves dispersing the powdered medicament in a propellant gas stream. For example, a pressurised canister of a liquefied gas may be connected to a vial containing the medicament. By releasing controlled amounts of gas from the canister into the vial, increments of the medicaments are ejected from the vial as a cloud of powder and may be inhaled by the user. The delivery device of the invention is advantageously arranged for sequential delivery of a multiplicity of individual inhalable doses. It is envisaged that, in use, one or two inhalable doses of medicament, each dose containing 50mg, may be administered up to three times daily.

The present invention also provides the use of a surface active phospholipid composition in the manufacture of a medicament for the treatment of asthma by respiratory administration of the medicament, the phospholipid composition being in finely divided solid form and comprising a first component which is capable of binding to lung tissue and a second component which is capable of enhancing the spreading of said first component, and in which said medicament is arranged for delivery of one or

more respirable individual doses, the or each dose comprising said first and second components in a total combined amount of at least 10mg.

Moreover, the invention provides the use of an SAPL composition in the manufacture of a medicament for the treatment of asthma by administration of the medicament to the patient's lungs, said medicament comprising a first component consisting of one or more phosphatidyl cholines and a second component consisting of one or more compounds selected from the group consisting of phosphatidyl glycerols, phosphatidyl ethanolamines, phosphatidyl serines, phosphatidyl inositols and chlorestyl palmitate, wherein said medicament is arranged for delivery to the patient's lungs in individual doses, such that each individual dose comprises at least 10mg of said first and second components in combination.

Further, the invention provides a combination treatment for use in the prevention or treatment of asthma comprising

(a) a medicament comprising a first phospholipid component which is capable of enhancing the spreading of said first component over an aqueous medium at 37°C, said composition being in the form of a powder in which at least 90% by weight of said first and second components consists of particles having a particle size of not greater than 30μm; and

b) antiasthma drug;

the ingredients (a) and (b) being arranged for administration in combination or separately, simultaneously or sequentially.

One form of dispenser according to the invention will now be described in detail, by way of illustration, with reference to the accompanying drawings, in which:

Fig. 1 is a side elevation of a delivery device;
Fig. 2 is a similar view, but shows its interior; and

In the drawings, a casing 1 is formed from two plastic mouldings 2 and 3 which snap together to form a container for a pressurised canister 4 and a vial 5. Canister 4 contains a low boiling liquid, preferably a hydrofluorocarbon such as HFA-134a or HFC-227, under sufficient pressure to maintain the propellant liquid at normal room temperature. Vial 5 contains the powdered medicament, such as "ALEC". Canister 4 has a release valve 6 which is received in a recess 7 so that finger pressure on the inverted end 8 of the canister will cause propellant to be released into a tube 9. Tube 9 is typically a hard plastics, e.g. pvc or polypropylene, tube of about 2-3mm outside diameter and about 0.5 to 2mm inside diameter. Tube 9 connects valve 6 with a fitting 10 and thence to a tube or needle 11 which extends into the vial 5. Vial 5 may be closed with a rubber seal which is penetrated by the tube or needle 11 and self-seals around the tube or needle. A second needle or tube 12 extends part way into the vial through the rubber seal in the neck of the vial and connects with a fitting 13. Fitting 13 discharges into a mouthpiece 14 which is a comfortable shape for the user to place in the mouth. When the patient is in need of medication, he places the mouthpiece 14 into his mouth and breaths and simultaneously depresses the canister 4. This causes a cloud of medicament to be dispensed into the patient's airways. Fittings 10 and 13 may be valves. Valves 10 may

be set to permit measured quantities of propellant to enter the vial. Similarly, valve 13 may be set to release when the pressure in the vial reaches a predetermined level. It will be appreciated that the dispenser can be used one-handed in an analogous manner to a conventional nebulizer.

In addition to the powdered phospholipid composition, the vial may incorporate other known pulmonary or respiratory medicaments such as salbutamol, Beclomethasone, corticosteroids, or other asthma drugs. It is, however, preferred to package the conventional asthma drug in the propellant canister or in a capsule interposed between the propellant container and the vial containing the phospholipid composition. In this way, the lungs and airways receive a cloud of phospholipid composition and an aerosol of the conventional drug sequentially or simultaneously. This combined therapy gives both quick relief and lasting protection as the film of phospholipid composition spreads over the lung tissue. Instead of packaging the phospholipid composition in a multi-use vial, it may be contained in a capsule, which may be a single use quantity, between the outlet from the propellant canister and the mouthpiece.

While the present invention has been described with particular reference to the treatment of human patients for asthma, it is possible that the invention may also be applicable to the treatment of other pulmonary diseases or conditions such as rhinnitis.

The medicament of the present invention may also be employed in the treatment of pulmonary conditions in other mammals. An example is reactive airway disease in horses.

As already mentioned, the finely divided ALEC for use in the device of the invention may be obtained by dissolving, filtering and vacuum-drying the components and

size-reducing the solid product so obtained. The size-reduced product has a relatively large respirable fraction. Thus, from a 4 litre inhalation from a vial containing 100mg of the size-reduced product, from 20 to 25mg was found to be of a size such that it would enter into the lung (determination using a Multi-Stage Impinger in accordance with the method described in European Pharacopoeia (supplement 1999), 2.9.18 (Aerodynamic assessment of fine particles).

The surface activity of phospholipid compositions may be determined as follows:

A 2cm x 2cm platinized grey dipping plate is heated to cherry red using the flame from a Bunsen burner or similar torch. The plate is suspended from an electronic balance capable of weighing up to 500mg.

To calibrate the apparatus, a small teflon dish is filled with distilled water at approximately 20°C (room temperature) and placed on a laboratory jack just beneath the dipping plate. The dish is then raised so that the dipping plate just breaks the surface of the water, evenly along the bottom edge. The meniscus drawn up the dipping place is used to set the display of the pen recorder of the electronic balance to read about $73mNm^{-1}$ (the air/water surface tension of water at 20°C). The Teflon dish is lowered, emptied, cleaned, dried and then filled with reagent grade methanol. The dipping plate is cleaned as described above. The dish is then raised so that the dipping plate just breaks the surface of the methanol, evenly along the bottom edge. The meniscus drawn up the dipping plate will cause the pen recorder to read about $22mNm^{-1}$ (the air/methanol surface tension of methanol at 20°C[1]). The Teflon dish is lowered and the dipping plate is cleaned as described above. A zero-reading should be

obtained for the cleaned plate alone (i.e. suspended in air).

To obtain a quantitative measure of the surface activity of a material, the Teflon dish is warmed to about 37°C, filled with water at not more than 37°C and placed on a laboratory jack just beneath the cleaned plate. The dish is then raised so that the dipping plate just breaks the surface of the water, evenly along the bottom edge. The meniscus drawn up the dipping plate will give a reading of about $70mNm^{-1}$ (the approximate air/water surface tension of warm water). The material is applied onto the surface of the water using a small spatula. The amount applied should be sufficient to ensure that a complete monolayer has been formed on the surface of the water, such that an excess (as small free-floating particles) can be observed. The surface tension should fall instantly, that fall being recorded by the pen recorder. Equilibrium surface tension readings are taken from the pen recorder after about 1 minute. The temperature of the water in the Teflon dish should be not less than 35° C immediately after the reading is taken.

The term "surface active" as used herein with reference to any composition for use in accordance with the invention means that the equilibrium surface tension, as measured in the above method, is at least 10% lower than the surface tension before the composition is applied to the water surface. In practice, the reduction in surface tension obtainable using the compositions may exceed 50%.

A component included in admixture with another material is to be understood as enhancing the spreading of the other material if, in carrying out the above method for determination of surface activity using the mixture and, separately, using the other material alone, the time taken

for the equilibrium surface tension to be reached is shorter for the mixture, as compared to the material alone.

The following Example illustrates the binding of a preferred phospholipid to the epithelium:

Example

Reagents

L-α-Phosphatidylcholine, 1,2-di[1-^{14}C]palmitoyl in Toluene:Ethanol (1:1 v/v), 114mCi/mmol, 50μCi in 2mL (CFA604 B36, Amersham)

L-α-Phosphatidylcholine, dipalmitoyl (C16:0) (P-6267, Sigma)

DL-α-Phosphatidyl-DL-glycerol, dipalmitoyl (C16:0) (P-5650, Sigma)

Egg Phosphatidylglycerol (Batch 24756, Macfarlan Smith, Ltd.)

Sodium Chloride, 0.9%, B.P. (Baxter Healthcare)

Calcium Chloride (C-4901, Sigma)

Toluene (T-4428, Sigma)

Ethanol, AnalaR (10107.7Y, BDH)

NCS-II Tissue Solubilizer, 0.5N Solution (NNCS-502), Amersham)

OCS Organic Counting Scintillant (NOCS104, Amersham)

In preparation for the dispersions in which the epithelium would be incubated, stock solutions of the phospholipid components were prepared on the first day of Run 1. These solutions were as follows:

L-α-DPPC, 2.4mg. mL^{-1} in toluene:ethanol, 1:1

DL-α-DPPG, 3.0mg. mL^{-1} in toluene:ethanol, 1:1

Egg PG, 3.0mg. mL^{-1} in toluene:ethanol, 1:1

All of the above solutions were stored at 4°C in glass vials, the threads of which were sealed with teflon tape to

minimise evaporation of the solvent. Each glass vial was then placed inside a second, tightly capped glass vial. These solutions were used for each of the five runs in the trial. A solution of 200mg. L^{-1} $CaCl^{2}$ in 0.9% saline was also prepared on the first day of Run 2 and was used in each of Runs 2 to 5.

Equipment

Special Ultrasonic Cleaner, Model Gl12 SPlG (Laboratory Supplies Co. Inc., Hicksville, N.Y., U.S.A.)
VF2 Vortex (IKA-Labortechnik)
Shaking Water Bath, Model TSB2-201-A (Thermoline Scientific Equipment, Smithfield, Australia)
Contherm Series Five, Fan Forced Oven (Contherm Scientific Ltd. Lower Hutt, N.Z.) TRI-CARB 2700TR Liquid Scintillation Analyser (Packard Instrument Co., Meriden, CT, U.S.A.)
Ultrasonic Cleaner, Model FXPI2 (Unisonics Pty. Ltd. Sydney, Australia)

Bronchial Epithelium

To provide a source of bronchial epithelium, porcine lungs were obtained from an abattoir within 24h of death. The lungs had been stored at 4°C since the time of death. The secondary bronchus was dissected from the right and/or left lungs. The exterior surface of the bronchus was trimmed of all lung tissue, and the bronchus was further cut into sections having a known surface area of bronchial epithelium (approximately 0.5cm x 0.5cm), leaving the epithelium and cartilage intact. The surface of the epithelium was rinsed with 0.9% saline to remove any mucus.

Where necessary sections of bronchial epithelium were stored in 0.9% saline at -20°C for 3 to 7 days until required for

use. The sections were thawed before use on the first day of each run.

For bronchial epithelium, a total of five runs were completed. Each run consisted of three groups, as follows:

1. DPPC only
2. DPPC + DPPG
3. DPPC+eggPG

Four dispersions were prepared on the first day of each run. All groups received both 20.5μL (3.3μg) of ^{14}C-L-α-DPPC and 5.5μL (13.2μg) of unlabelled L-α-DPPC from the stock solutions. In addition, Group 2 received 5.5μL (16.5μg) DL-α-DPPG, while the same quantity of egg PG was added to Group 3. In Groups 2 and 3, the ratio of total DPPC to PG was 1:1. The phospholipid component was mixed with 6.6ml of 0.9% saline for Groups 1, 2, and 3. All of the above listed volumes were used when there were two sections of epithelium in each treatment group. When the number of sections was increased, the volumes of all components were increased accordingly, keeping all quantities in the same proportions as above. Table 1 summarises the additives to the incubation mixtures.

Table 1. Components of Incubation Dispersions

Group	Saline	^{14}C-L-α-DPPC	L-α-DPPC	DL-α-DPPC	Egg PG
1	X	X	X		
2	X	X	X	X	
3	X	X	X		X

To solubilise the phospholipid components in the aqueous medium, each of the four incubation dispersions was sonicated for 45min, then vortexed to mix for 1min.

From each dispersion, two lots of 2.8mL were transferred to two glass vials. A single section of epithelium was incubated in each of these dispersions, so that there were four groups of two sections of bronchial epithelium in each group. Bronchial epithelium was taken from a single pig on any given day of incubation. Incubation was at 37°C for 24h in a shaking water bath.

Aliquots of the Group 1 dispersion were transferred to glass scintillation vials and incubated at 37°C in an oven for the 24h. These aliquots were used as the standards for the calibration curve. Matching aliquots from the other group dispersions were also taken, and the ß-counts from these were compared with those from the group 1 dispersion as a check that all dispersions contained the same quantity of DPPC.

On the second day of each run, the sections of epithelium were removed from the incubation dispersions and were each rinsed 20 times with 0.9% saline, warmed to 37°C in a water bath, to remove any loosely adhering phospholipid. Care was taken not to mechanically disturb the mucosal surface of epithelium. Each section of bronchial epithelium was then removed from the attached cartilage. The sections of epithelium were further cut into smaller pieces to aid the digestion of the tissue by the solubilising agent which was added in a volume of 1 .5mL to the epithelium in scintillation vials. The same volume of solubiliser was added to each of the standard aliquots and to a blank. All vials were gently shaken to mix the contents and were warmed to 55°C in a fan-forced convection oven overnight (18-20h).

On the third day of each run, 10mL of organic counting scintillant were added to each scintillation vial, and these were vortexed to mix for 30s.

The ß-counts of each sample and standard were measured using a liquid scintillation analyser. A second count was conducted within 7h of the first count. If the two counts were similar, only the first count was used to construct the line of calibration and to quantify the samples.

From the line of calibration, the mass of ^{14}C-DPPC adsorbed to each section of epithelium was calculated. To calculate the mass of total DPPC adsorbed to each section, the mass of ^{14}C-DPPC was multiplied by 5 since the quantity of ^{14}C-DPPC in each of the dispersions was 1/5 of the total amount of DPPC. The result is expressed in Table 2 as the total amount of DPPC adsorbed per cm^2 of epithelium.

The results in Table 2 show that increased binding of DPPC to bronchial epithelium is observed in the presence of DPPG, but that the extent of binding is improved still further where Egg PG is used instead of DPPG.

Table 2.

Total DPPC Adsorbed to Bronchial Epithelium ($\mu g/cm^2$)

	DPPC	DPPC:DPPG,1:1	DPPC:Egg PG,1:1
	0.341	0.501	0.878
	0.299	0.321	0.743
	0.219	0.214	0.472
	0.116	0.263	0.731
	0.276	0.378	0.705
	0.280	0.494	0.529
	0.528	0.355	0.836
	0.192	0.419	0.792
	0.340	0.294	0.986
	0.321	0.362	0.791
n	10	10	10
Mean	0.291	0.360	0.746
SD	0.110	0.093	0.153

Claims

1. A delivery device for administering to a patient by inhalation a medicament for the prevention or treatment of asthma, the delivery device containing a medicament comprising a surface active phospholipid composition which is in finely divided solid form and comprises a first component which is a phospholipid capable of binding to lung tissue and a second component which is a phospholipid capable of enhancing the spreading of said first component, the delivery device being arranged for delivery of at least one individual inhalable dose, the or each individual dose comprising said first and second components in a combined amount of 10mg.

2. A delivery device for administering to a patient by inhalation a medicament for the prevention or treatment of asthma, the delivery device containing a medicament comprising a first component consisting of one or more phosphatidyl cholines and a second component consisting of one or more compounds selected from the group consisting of phosphatidyl glycerols, phosphatidyl ethanolamines, phosphatidyl serines, phosphatidyl inositols and chlorestyl palmitate, the delivery device being arranged for delivery of at least one individual inhalable dose, the or each individual dose comprising said first component and said second component in a combined amount of at least 10mg.

3. A delivery device as claimed in claim 1 or claim 2, which is arranged for delivery of at least one individual inhalable dose, the or each individual dose comprising said first and second components in a combined amount of 25mg.

4. A delivery device as claimed in claim 3, which is arranged for delivery of at least one inhalable individual dose, the or each dose comprising said first and second components in a combined amount of at least 40mg.

5. A delivery device as claimed in any one of claims 1 to 4, which is arranged for sequential delivery of a multiplicity of inhalable doses.

6. A delivery device as claimed in claim 5, which comprises actuation means for actuating the device to deliver a dose.

7. A delivery device as claimed in any one of claims 1 to 6, which comprises said first component and said second component in a weight ratio of from 1:9 to 9:1.

8. A delivery device as claimed in claim 7, in which the proportion by weight of said first component exceeds that of said second component.

9. A delivery device as claimed in claim 8, in which said first component and said second component are present in a weight ratio of from 6:4 to 8:2.

10. A delivery device as claimed in any one of claims 1 to 9, in which the medicament comprises a phosphatidyl glycerol.

11. A delivery device as claimed in claim 10, in which the phosphatidyl glycerol comprises one or more diacyl phosphatidyl glycerols, of which at least a proportion of the acyl groups are unsaturated.

12. A delivery device as claimed in any one of claims 1 to 11, in which the first component of the medicament comprises one or more compounds selected from the group consisting of diacyl phosphatidyl cholines.

13. A delivery device as claimed in claim 12, in which the medicament comprises dipalmitoyl phosphatidyl choline.

14. A delivery device as claimed in any one of claims 1 to 13, in which the medicament further comprises one or more respiratory drugs selected from the group consisting of $ß_2$-agonists, steroids, cromones, antimuscarinic drugs and leukotriene receptor antagonists.

15. A delivery device as claimed in claim 14, in which the medicament comprises one or more said respiratory drugs in an amount of up to 10 parts by weight per hundred parts by weight of said first and second components in combination.

16. A delivery device as claimed in claim 15, in which the medicament comprises one or more said respiratory drugs in an amount of up to one part by weight per hundred parts by weight of said first and second components in combination.

17. A delivery device as claimed in any one of claims 1 to 16, in which said first and second components are in micronised form.

18. A delivery device as claimed in any one of claims 1 to 17, in which said first and second components have a median particle size not exceeding 10μm.

19. A delivery device as claimed in any one of claim 1 to 17, in which said first and second components have a median particle size not exceeding 5μm.

20. A delivery device as claimed in claim 18, in which said first and second components have a median particle size of less than 3μm.

21. A delivery device substantially as described herein.

22. A pack comprising at least one dosage unit for use in a delivery device according to any one of claims 1 to 20.

23. A pack as claimed in claim 21, which is in the form of a blister pack comprising a multiplicity of individual dosage units.

24. The use of a phospholipid composition in the manufacture of an inhalable medicament for the treatment of asthma, the phospholipid composition being surface active and comprising a first component which is capable of binding

to lung tissue and a second component which is capable of enhancing the spreading of said first component, and in which said medicament is arranged for delivery of one or more inhalable individual doses, the or each dose comprising said first and second components in a total combined amount of at least 10mg.

25. The use of a phospholipid composition in the manufacture of an inhalable medicament for the treatment of asthma, said phospholipid composition comprising a first component consisting of one or more phosphatidyl cholines and a second component consisting of one or more compounds selected from the group consisting of phosphatidyl glycerols, phosphatidyl ethanolamines, phosphatidyl serines, phosphatidyl inositols and chlorestyl palmitate, and wherein said medicament is arranged for delivery to the patient's lungs in one or more respirable individual doses, the or each individual dose comprising said first and second components in a total combined amount of at least 10mg.

26. Use as claimed in claim 23 or claim 24, in which the medicament is arranged for delivery in individual doses each comprising at least 25mg of said first and second components.

27. Use as claimed in claim 25, in which the medicament is arranged for delivery in individual doses each comprising at least 40mg of said first and second components.

28. Use as claimed in any one of claims 23 to 26, in which the medicament is as defined in any one of claims 7 to 19.

29. A method of treatment as asthma, comprising administering to a patient by inhalation at least one dose of a medicament as defined in any one of claims 24 to 28,

said dose comprising said first and second components in a total combined amount of at least 10mg.

30. A combination treatment for use in the prevention or treatment of asthma comprising

(a) a medicament comprising a first phospholipid component which is capable of binding to lung tissue and a second component which is capable of enhancing the spreading of said first component over an aqueous medium at 37°C, said composition being in the form of a powder in which at least 90% by weight of said first and second components consists of particles having a particle size of not greater than 10μm; and

(b) an antiasthma drug;

the ingredients (a) and (b) being arranged for administration in combination or separately, simultaneously or sequentially.

31. A combination treatment as claimed in claim 30, in which ingredient(a) consists of one or more phosphatidyl cholines and the second component consists of one or more compounds selected from the group consisting of phosphatidyl glycerols, phosphatidyl ethanolamines, phosphatidyl serines, phosphatidyl inositols and chlorestyl palmitate,

32. A combination treatment as claimed in claim 30 or claim 31, in which ingredient (a) is a medicament as further defined in any one of claims 7 to 19.

33. A combination treatment as claimed in any one of claims 30 to 32, in which ingredient (b) comprises a $ß_2$-agonist.

34. A combination treatment as claimed in any one of claims 30 to 33, in which ingredient (b) comprises a steroid.

35. A combination treatment as claimed in any one of claim 30 to 34, in which ingredient (b) comprises a cromone.

36. A combination treatment as claimed in any one of claims 30 to 35, in which ingredient (b) comprises a leukotriene receptor antagonist.

37. A combination treatment as claimed in any one of claims 30 to 36, in which ingredient (b) comprises an antimuscarinic drug.

Improvements in and relating to treatment of respiratory conditions

Abstract

A device for use in treating asthma and other respiratory conditions containing a medicament comprising a surface active phospholipid composition in the form of a fine powder. The device is arranged to administer a specified dose to the lungs by inhalation. A composition comprises dipalmitoyl phosphatidyl choline and phosphatidyl glycerol.

Fig. 1

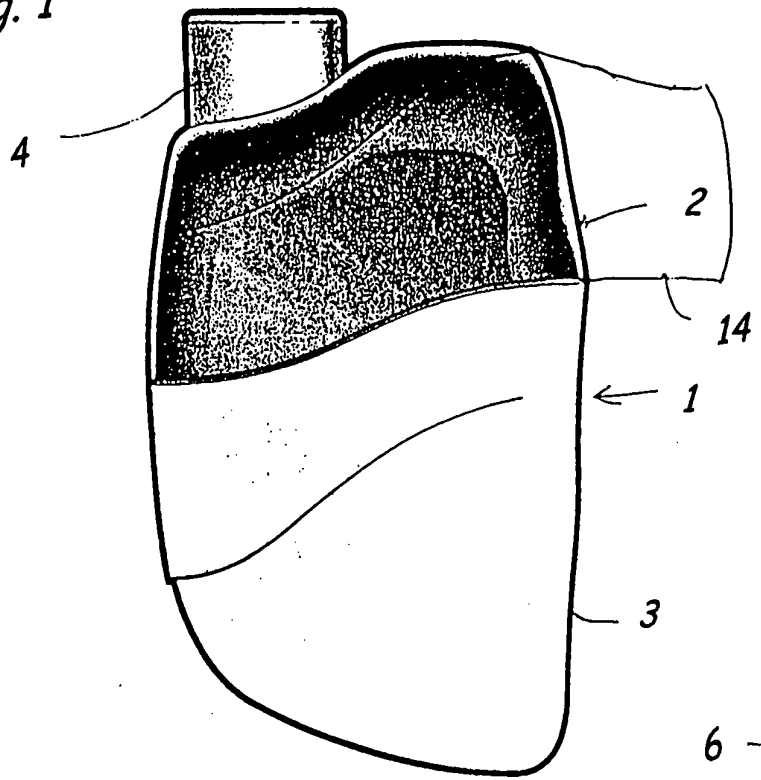
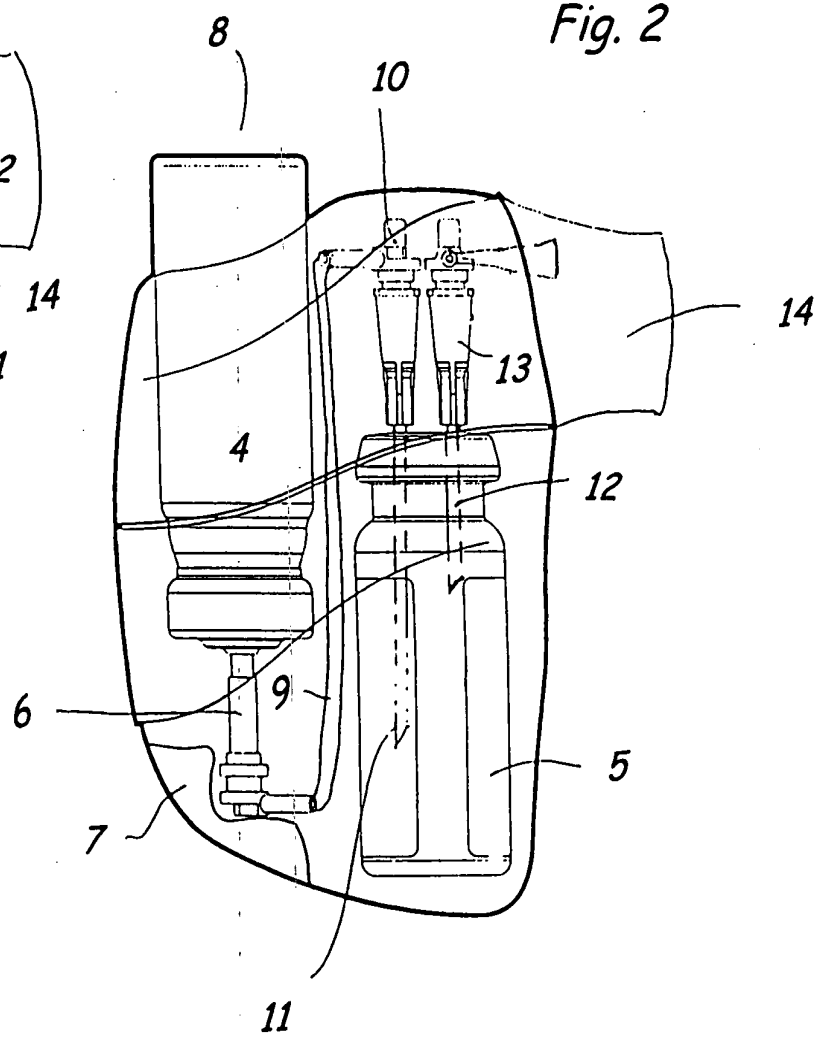


Fig. 2



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